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Forum Editorial

Recent Advances in Understanding the Unfolded Protein Response

MARTIN SCHRÖDER[1] and KENJI KOHNO[2]

THE UNFOLDED PROTEIN RESPONSE (UPR), a recently discovered network of signal transduction pathways from the endoplasmic reticulum (ER) to the nucleus, has been brought to the limelight by a flurry of recent articles establishing its central role in many diseases, ranging from the metabolic syndrome to neurological or conformational diseases caused by formation of toxic intracellular protein aggregates. In the past few years, our understanding of how the UPR functions on a molecular level has increased considerably, as has our understanding of the role of the UPR in normal physiology and in pathophysiologic situations. Nevertheless, important questions remain to be answered, of which some are as old as research into the UPR. This forum issue on the UPR summarizes our current understanding of the UPR and highlights its importance in human disease as well as still outstanding questions that remain to be answered.

ACTIVATION OF THE UPR

The UPR is a classic homeostatic feedback loop, whose physiologic role is to maintain homeostasis of the ER, or in other words, the balance between the folding demand posed onto the ER by the influx of newly synthesized, unfolded polypeptide chains and the folding capacity of the ER-resident protein folding machinery. If this balance is perturbed, at least three classes of transmembrane proteins, basic leucine zipper (bZIP) transcription factors synthesized as type II transmembrane precursors, of which the best studied are activating transcription factor (ATF) 6α and ATF6β/cyclic adenosine monophosphate (cAMP) response element–binding protein (CREB)-related protein (CREB-RP)/G13, the type I transmembrane protein kinase eukaryotic translation initiation factor 2α (eIF2α) kinase 3 (EIF2AK3)/pancreatic eIF2α kinase (PEK)/double-stranded ribonucleic acid (RNA)-activated protein kinase (PKR)-like ER kinase (PERK), and the type I transmembrane protein kinase-endoribonucleases ER to nucleus signaling (ERN) 1α/inositol-requiring (IRE) 1α and ERN1β/IRE1β, are activated to transduce the ER stress signal across the ER membrane. The ER-resident protein folding and quality control machinery regulates the oligomerization status of these protein kinases *via* association of the ER-resident molecular chaperone heavy chain–binding protein (BiP)/glucose-regulated protein of 78 kDa (GRP78)/karyogamy 2 protein (Kar2p) with their ER luminal domains. Kohno (17) summarizes new research that characterizes signals that activate the ER stress sensors IRE1 and PERK, and how BiP regulates these two protein kinases. BiP also binds to the ER luminal domain of ATF6 in unstressed cells and is released from ATF6 during ER stress, triggering translocation of ATF6 to the Golgi complex, where its cytosolic bZIP transcription factor domain is proteolytically released from the Golgi membrane by successive cleavage by site 1 and 2 proteases (S1P and S2P) (33). The activation mechanism of ATF6 and its distant paralogs CREB3, CREB4, CREB-H, box B–binding factor (BBF) 2, and old astrocyte specially induced substance (OASIS) and our current knowledge about the specific roles of these transcription factors in the UPR is discussed by Bailey and O'Hare (2).

SIGNAL-TRANSDUCTION MECHANISM

The protein kinase PERK regulates translation initiation and the activity of the bZIP transcription factor nuclear factor erythroid 2 (NF-E2)–related factor 2 (NRF2). NRF2, as a heterodimer with the bZIP transcription factor ATF4, activates an antioxidant response (10). About 25% of all reactive oxygen species are formed as a byproduct of oxidative protein folding

[1]School of Biological and Biomedical Sciences, Durham University, South Road, Durham, England.
[2]Laboratory of Molecular and Cell Genetics, Graduate School of Biological Sciences, Nara Institute of Science and Technology (NAIST), Takayama, Ikoma, Nara, Japan.

in the ER (9, 29). Malhotra and Kaufman (20) discuss the interaction between ER stress and oxidative stress in mammalian cells. Kim-Han and O'Malley (15) describe how oxidation of the protein disulfide isomerase (PDI) ER protein of 57 kDa (ERp57)/GRP58 by the parkinsonian mimetic 6-hydroxydopamine results in its deposition in aggresome-like structures, thus demonstrating a link between inactivation of an ER-resident protein disulfide isomerase and Parkinson's disease. Phosphorylation of eIF2α by PERK attenuates general translation to decrease the influx of newly synthesized, unfolded proteins into the ER (8). Inhibition of translation also results in clearance of short-lived proteins from the cell. Clearance of inhibitors of the transcription factor (IκBs) activates nuclear factor κB (NF-κB) and a systemic inflammatory response. Clearance of D-type cyclins arrests ER-stressed cells in G_1 phase. A complex regulatory mechanism also promotes preferential translation of the messenger RNA (mRNA) for the bZIP transcription factor ATF4 when eIF2α is phosphorylated. Transcriptional activation of the *CHOP/CHOP-10/GADD153* gene by ATF4 is a major route for induction of apoptosis in the UPR. The mechanism and role of translational control in the UPR is discussed in detail by Wek and Cavener in this issue (31).

The most intriguing signal-transduction mechanism in the UPR is the transfer RNA (tRNA)-like splicing of *homologous to ATF/CREB1* (*HAC1)* and *hepatocarcinogenesis-related transcription factor* (*HTF)/tax-responsive element–binding protein 5* (*TREB5)/X box–binding protein* (*XBP-1)* mRNAs by IRE1. Much of our knowledge on this unconventional mRNA splicing mechanism, which so far seems to be unique to the UPR, comes from studies in yeast. As in tRNA splicing, endonucleolytic cleavage of *HAC1* mRNA generates a 2′,3′-cyclic phosphate end, which is ligated in an adenosine triphosphate (ATP)-consuming reaction to a 5′-hydroxyl group of the second *HAC1* exon by tRNA ligase (6). A mammalian *XBP-1* ligase, or even a tRNA ligase, has not yet been cloned, despite longstanding biochemical evidence for the existence of two different tRNA ligase activities in mammalian cells (18, 37). Controversial also is the question whether *HAC1* and *XBP-1* mRNA splicing is a nuclear or cytosolic event. Earlier studies localized tRNA ligase to the nucleus, which is also the site of tRNA endonuclease (4). More recent work provided evidence for cytosolic localization of tRNA ligase (14) and tRNA endonuclease subunits (36) in yeast. *HAC1* splicing was observed in polyribosomes, suggesting a cytosolic localization of the splicing reaction (25). Similarly contradictive is the experimental evidence for the localization of *XBP-1* splicing. IRE1α was localized biochemically to the inner nuclear envelope (19), but more recent studies point toward cytosolic *XBP-1* splicing (1). The evidence in favor of nucleoplasmic or cytosolic splicing is discussed by Yoshida (34).

PHYSIOLOGIC ROLE OF THE UPR

The higher eukaryotic UPR is thought to occur in at least three phases, a folding only phase, a folding and degradation phase, and finally, if ER homeostasis cannot be restored, apoptosis. In the folding only phase, ATF6 induces the synthesis of ER-resident molecular chaperones. Activation of XBP-1 lags behind proteolytic activation of ATF6 and induces the folding and degradation phase by activating expression of genes encoding proteins involved in ER-associated protein degradation (ERAD) (35). Recent work has shown that ERAD extends to co-translational degradation of newly synthesized proteins whose translocation into the ER is delayed (22), and even to co-translational degradation of mRNAs at the ER membrane by IRE1 (12). Kincaid and Cooper (16) discuss the role of ERAD in the adaptive phase of the UPR. If activation of adaptive responses to ER stress cannot return the ER to homeostasis, the UPR initiates apoptosis (32). Apoptosis is initiated *via* Ca^{2+} efflux from the ER, activation of a caspase cascade, and regulation of B-cell leukemia/lymphoma 2 (Bcl-2) family protein interactions at the ER membrane. Interestingly, protective arms of the UPR also transduce apoptotic signals, and it is still not well understood, how, and whether, signaling specificity in these pathways is established. Hetz (11) summarizes recent research showing that Bcl-2 family proteins regulate Ca^{2+} homeostasis, ER morphology, autophagy, and UPR signaling in addition to ER stress–induced apoptosis.

Of outstanding interest is the role of the UPR in human disease. Conformational diseases are caused by aberrant protein conformation, resulting in formation of protein aggregates. Important questions are which protein conformations elicit an UPR, and which cellular functions are affected by these conformations. Extracellular and possibly intracellular accumulation of amyloidic fibrils (*i.e.*, of amyloid β in Alzheimer's disease) is thought to be toxic and elicits a UPR (13, 30). Amyloid fibrils adopt a well-ordered β-sheet structure. Recent evidence suggests that not the fibrils themselves, but their precursors or "protofibrils" are cytotoxic, possibly interfering with proper membrane and protein function, especially sequestering and inhibiting chaperones (21). Chafeker *et al.* (3) show that in the case of extracellular amyloid β, it is the oligomeric, and not the fibrillar, form of amyloid β that activates the UPR. Thus, intracellular formation of amyloid β may interfere with the cellular chaperone machinery or integrity of the membranes of the secretory pathway. Alternatively, activation of the UPR is a compensatory mechanism to counteract perturbations at the plasma membrane caused by interaction of oligomeric amyloid β with phospholipids and proteins of the plasma membrane.

More surprisingly, the ER and the UPR have also been identified as central players in nutrient sensing (27, 28) and metabolic disease. Mouse knockout models have shown that disruption of UPR signaling results in pancreatic β-cell death and development of type II diabetes (7, 26). More recent work has shown that ER stress and UPR signaling confer peripheral insulin resistance (23). The central role of the UPR in pancreatic β-cell function is discussed by Urano (5). Hypoxia, as experienced by growing tumors and ischemia, is another example of nutritional perturbations resulting in activation of the UPR. Roberts *et al.* (24) critically assess the role of the UPR and the heat shock response in cerebral ischemia and reperfusion. By summarizing the progress made and identifying unanswered open questions, this forum issue on the UPR illustrates the bright future for research into the UPR and its physiologic and pathophysiologic roles.

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ABBREVIATIONS

ATF, activating transcription factor; ATP, adenosine triphosphate; BBF2, box B–binding factor; Bcl-2, B-cell leukemia/lymphoma 2; BiP, heavy chain–binding protein; bZIP, basic leucine zipper; cAMP, cyclic adenosine monophosphate, C/EBP, CCAAT enhancer–binding protein; CHOP, C/EBP homologous protein; CRE, cAMP response element; CREB, CRE-binding protein; CREB-RP, CREB-related protein; eIF2α, eukaryotic translation initiation factor 2α; EIF2AK3, eIF2α kinase 3; ER, endoplasmic reticulum; ERAD, ER-associated protein degradation; ERN1, ER to nucleus signaling 1; ERp57, ER protein of 57 kDa; GADD153, growth arrest and DNA damage gene 153; GRP78, glucose-regulated protein of 78 kDa; HAC1, homologous to ATF/CREB1; HTF, hepatocarcinogenesis-related transcription factor; HTLV-1, type 1 human T-cell leukemia virus; IκB, inhibitor of NF-κB; IRE1, inositol-requiring 1; Kar2p, karyogamy 2 protein; mRNA, messenger RNA; NF-E2, nuclear factor erythroid 2; NF-κB, nuclear factor κB; NRF2, NF-E2–related factor; OASIS, old astrocyte specifically induced substance; PDI, protein disulfide isomerase; PEK, pancreatic eIF2α kinase; PERK, PKR-like ER kinase; PKR, double-stranded RNA-activated protein kinase; S1P, site 1 protease; S2P, site 2 protease; RNA, ribonucleic acid; Tax, 40-kDa transactivator protein of HTLV-1; TREB5, Tax-responsive element–binding protein 5; tRNA, transfer RNA; UPR, unfolded protein response; XBP-1, X box–binding protein.

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Address reprint requests to:
Martin Schröder
School of Biological and Biomedical Sciences
Durham University
South Road
Durham DH1 3LE, U.K.

E-mail: martin.schroeder@durham.ac.uk

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